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Effects of Hypophysectomy and Replacement Therapy with Several Hormones on Plasma Sodium Concentrations in Bullfrog Tadpoles

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ABSTRACT—Hypophysectomy significantly decreased plasma sodium concentrations in bullfrog tadpoles kept in low-sodium media (2.5 meq/liter). A study on replacement therapy revealed that administration of tadpole pituitary homogenate corrected this hyponatremia in hypophysectomized tadpoles. These results show that in a low-sodium environment where the tadpoles live in nature, the pituitary gland is important for plasma sodium regulation. AVT and ovine prolactin did not maintain normal plasma sodium levels in hypophysectomized tadpoles, whereas ACTH and corticosteroids (especially cortisol) restrained the sodium decrease. Therefore, in pre-metamorphic tadpoles it seems that the pituitary-adrenal axis plays an important role in plasma sodium regulation.

INTRODUCTION

Among the lower vertebrates, the amphibians, as a group, have been studied extensively with regard to their mechanisms of osmotic and ionic regulation. It is well known that the skin of amphibians can actively take up salt from the surrounding aqueous environment. In these animals, corticosteroids especially aldosterone, and neurohypophysial hormone, arginine vasotocin (AVT), generally show potent natriferic and osmotic actions [see 1, 2]. It is known that tadpoles also actively transport sodium and chloride ions into their body fluids from a low-concentration external solution [3–6]. The integument of tadpoles, however, has been thought to be inessential for absorption of ions from the environment [7–9]. *In vitro* studies have shown that the main organ for active uptake of ions in tadpoles is not the skin but the gills [4, 10]. On the other hand, in unfed freshwater fish, accumulation of sodium depends almost entirely on the active uptake of sodium across the gill epithelium. It is also known that hypophysectomy results in an increased rate of sodium loss, and that in many cases, this can be prevented by injection of prolactin [reviewed by 11]. However, injection of ovine prolactin into normal tadpoles acclimated to low aqueous calcium and sodium or high aqueous calcium and sodium does not produce consistent hypernatremia [12], and it has been observed that ovine prolactin does not correct hyponatremia in hypophysectomized tadpoles [13]. In tadpoles, therefore, the role of prolactin in plasma sodium metabolism seems to be minor.

The present study was undertaken to investigate hypernatremic hormone(s) in bullfrog tadpoles, with special reference to the effects of replacement therapy with both pituitary gland and pituitary hormone and corticosteroid on plasma sodium concentration in hypophysectomized tadpoles.

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MATERIALS AND METHODS

Tadpoles of the bullfrog, *Rana catesbeiana* Shaw, at T-K stages VI to XIV were obtained from commercial dealers in Niigata [14]. They were kept for at least 1 week in low-calcium and low-sodium water (Ca: 0.85 meq/liter, Na: 2.5 meq/liter) at room temperature and maintained unfed during the experiments. Tadpoles were anesthetized in 0.05% MS 222 (m-aminobenzoic acid ethylester methanesulfonate, Sankyo) prior to manipulation, hormone injection and blood sampling. The procedures of blood sampling and the method of plasma sodium determination have been described previously [12]. All data are presented as means±SE and statistical analysis of differences in means was performed using Student's t-test.

Hypophysectomy and autotransplantation or pituitary homogenate injection

Hypophysectomy and autotransplantation or pituitary homogenate injection were done by the methods reported previously [12, 15]. In the autotransplantation experiment, tadpoles were hypophysectomized and then the removed pituitary gland was transplanted under the skin of the head region of the same individual (HYPX+autotransp.). A piece of muscle from each tadpole was transplanted into sham-transplanted tadpoles (HYPX+m. transp.). In the pituitary homogenate injection study, hypophysectomized tadpoles were injected intraperitoneally with a homogenate of tadpole pituitary gland. Each animal received a homogenate of 2 pituitary glands per injection daily for 6 days.

In these experiments, treated tadpoles were maintained in low-sodium water (Na: 2.5 meq/liter) for one more week and then sacrificed for blood sampling.

Hypophysectomy and hormonal treatment

In these experiments, hypophysectomized tadpoles were divided into several groups and maintained in low-sodium water (Na: 2.5 meq/liter). Then the effects of certain hormones were examined. The following hormones were used: ovine prolactin (NIH-p-S8), aldosterone (Sigma), ACTH (Armour Pharmaceutical Co.), corticosterone (Sigma), cortisol (Merck Sharp & Dohme), and AVT (Sandoz). These hormones were dissolved in 0.6% saline and/or 95% ethanol. Injections (20–25 μl/animal) were made with a Hamilton microsyringe into the lymph sinus beneath the skin of the back, passing through the tail muscles to prevent leakage. Injections were started one day after the operation. Each group received one of the above-mentioned hormones or the combinations of them daily for 6 days. Two series of experiments were performed.

RESULTS

Hypophysectomy followed by autotransplantation or injection of pituitary homogenates

The results obtained in these experiments are shown in Table 1. It was confirmed that hypophysectomy caused significant hyponatremia, showing that the pituitary gland is important for plasma sodium regulation in bullfrog tadpoles. There was a significant difference between the plasma sodium concentration in the sham operation group and that in the HYPX+muscle transplant group ($P<0.001$). In the second experiment,

TABLE 1. Effect of hypophysectomy and replacement therapy on plasma sodium in bullfrog tadpoles

Experiment	Treatment	Number	Time after treatment	Na (meq/liter)	Significance compared to: sham operation	Significance compared to: HYPX[a]+m. transp. or saline
	sham operation	9	1 week	97.9±1.4[b]	—	P<0.001
1[c]	HYPX+m. transp.	10	1 week	87.5±1.3	P<0.001	—
	HYPX+autotransp.	9	1 week	92.2±2.0	P<0.05	NS
	sham operation	6	1 week	96.0±1.0	—	P<0.001
2	HYPX+saline	9	1 week	86.8±1.4	P<0.001	—
	HYPX+pituitary homogenate	12	1 week	93.0±1.7	NS	P<0.05

[a] Abbreviations used: HYPX, hypophysectomy; m. transp., muscle transplantation; saline, saline injection.
[b] Values are means±SE.
[c] Number of experiments conducted: 1, HYPX and autotransplantation; 2, HYPX and injection with pituitary homogenates.

plasma sodium concentration in the sham operation group was significantly higher than that in the HYPX+saline group (P<0.001). The plasma sodium concentration in the HYPX+pituitary homogenate group was also significantly higher than that in the HYPX+saline group (P<0.05).

Hypophysectomy and hormonal treatment

In the first experiment, the effects of different doses of a hormone or a combination of two hormones were examined on plasma sodium concentration in hypophysectomized tadpoles. Table 2 shows the results obtained from this experiment. Plasma sodium concentration in the sham operation+saline group was significantly different from those in the HYPX+saline, HYPX+AVT (0.02 ng/g and 0.2 ng/g) and HYPX+ovine prolactin (5 μg/g) groups (P<0.05). Injections of ACTH (50 ng/g), cortisol (0.5 μg/g, 5 μg/g), ACTH and ovine prolactin in combination, or aldosterone (50 ng/g) into hypophysectomized tadpoles produced significantly higher plasma sodium concentrations than those in hypophysectomized tadpoles (P<0.05), whereas no statistically significant difference was found between the HYPX+saline group and the other experimental groups. In the second experi-

TABLE 2. Effect of hypophysectomy and hormonal replacement therapy on plasma sodium in bullfrog tadpoles

Treatment	Number	Na (meq/liter)	Significance compared to: sham operation +saline	HYPX +saline
sham operation+saline	14	99.9±1.0[a]	—	P<0.05
HYPX+saline	10	97.0±0.5	P<0.05	—
HYPX+ACTH (50 ng/g)	10	100.4±0.8	NS	P<0.01
HYPX+ACTH (500 ng/g)	10	99.3±1.1	NS	NS
HYPX+aldosterone (5 ng/g)	8	99.1±2.1	NS	NS
HYPX+aldosterone (50 ng/g)	12	100.2±0.9	NS	P<0.01
HYPX+corticosterone (0.5 μg/g)	12	97.5±0.9	NS	NS
HYPX+corticosterone (5 μg/g)	10	98.8±1.2	NS	NS
HYPX+cortisol (0.5 μg/g)	12	99.8±1.1	NS	P<0.05
HYPX+cortisol (5 μg/g)	10	100.8±1.1	NS	P<0.05
HYPX+oPRL (5 μg/g)	9	95.9±1.4	P<0.05	NS
HYPX+oPRL (5 μg/g)+ACTH (150 ng/g)	12	100.8±0.9	NS	P<0.01
HYPX+AVT (0.02 ng/g)	12	95.7±1.2	P<0.05	NS
HYPX+AVT (0.2 ng/g)	12	96.9±1.0	P<0.05	NS

[a] Values are means±SE.

TABLE 3. Effect of hypophysectomy and hormonal replacement therapy on plasma sodium in bullfrog tadpoles

Treatment	Number	Na (meq/liter)	Significance compared to: sham operation +saline	HYPX +saline
sham operation+saline	10	98.4±0.8[a]	—	P<0.05
HYPX+saline	8	91.0±2.4	P<0.05	—
HYPX+ACTH (50 ng/g)	8	96.2±2.0	NS	NS
HYPX+aldosterone (50 ng/g)	5	94.8±3.0	NS	NS
HYPX+cortisol (5 μg/g)	10	100.0±1.8	NS	P<0.01
HYPX+oPRL (5 μg/g)	8	94.4±2.8	NS	NS

[a] Values are means±SE.

ment, ACTH, aldosterone and ovine prolactin failed to maintain normal plasma sodium concentrations. However, cortisol corrected the plasma sodium concentration after hypophysectomy ($P < 0.01$). These results are summarized in Table 3.

DISCUSSION

In bullfrog tadpoles kept in low-sodium water (Na: 2.5 meq/liter), the previous observation that hypophysectomy brought about a significant decrease in plasma sodium concentration was confirmed [12, 13]. In the replacement therapy study, administration of tadpole pituitary homogenate corrected the hyponatremia and pituitary grafts partially prevented this in hypophysectomized tadpoles. These results suggest that the pituitary gland is important for maintaining plasma sodium concentrations in bullfrog tadpoles. The next question to be answered was which pituitary hormones are important for the control of plasma sodium concentration in bullfrog tadpoles.

In the present study, AVT was not able to maintain a normal plasma sodium level after hypophysectomy. Bentley and Greenwald [16] reported that the pituitary gland of the bullfrog tadpole contains only about one-fifth of the neurohypophysial peptides (activity/kg body weight) present in the adult frog. It is also reported that anuran tadpoles exhibit little or no response to AVT, although AVT is an antidiuretic and natriferic hormone in adult amphibians [8, 16]. Therefore, the present result might be explained by assuming that AVT administration is not sufficient for maintaining a normal plasma sodium level and/or AVT might not be a potent agent for the control of hydromineral balance in young tadpoles. In the present study, prolactin was also unable to restore plasma sodium concentrations in hypophysectomized tadpoles. This result is consistent with the previous report [13]. Brown and Brown [17] suggested that the major role of prolactin in amphibians might be water retention. If this is so in bullfrog tadpoles, it is possible that the increase in plasma sodium concentration caused by prolactin treatment might be masked by hemodilution. On the other hand, Clemons and Nicoll [18] reported that the circulating plasma level and pituitary content of prolactin were increased significantly during metamorphic climax in bullfrog tadpoles. It was also shown that during the climax stage, prolactin is involved in regulation of the active sodium transport system in the ventral skin of bullfrog tadpoles [19, 20]. The bullfrog tadpoles used in the present study were pre-metamorphic larvae (stages VI-XIV). Therefore, there are two possible explanations for the minimal effect of prolaction on sodium regulation in young tadpoles. One is that prolactin secretion is insufficient in young tadpoles and the other is that the target systems of prolactin sodium transport might be undeveloped. The latter concept is probably true in the case of ACTH (presumably acting through the adrenal gland), since Krug *et al.* [21] showed that a single injection of ACTH failed to elevate the serum level of aldosterone, corticosterone and cortisol in pre-metamorphic larvae (stages X-XIV), whereas the same treatment caused a striking increase in the levels of corticosterone and cortisol in more advanced tadpoles (stages XV-XIX). In the present study, however, it is possible that long-term ACTH treatment might have stimulated the production of corticosteroid in young tadpoles.

It is generally accepted that aldosterone and corticosterone are produced in the interrenal organ, and that corticosteroids, especially aldosterone, are involved in the regulation of electrolytes in adult amphibians. However, little information is available on the influence of corticosteroids on electrolyte regulation in larval anurans. Krug *et al.* [21] reported that serum aldosterone was maintained at fairly low levels in bullfrog tadpoles until a signifcant increase occurred in the metamorphic climax stage. In the present study, injection of aldosterone was inconsistent in restoring hyponatremia caused by hypophysectomy. Therefore, aldosterone might not be a major sodium-regulating agent in pre-metamorphic bullfrog tadpoles. Krug *et al.* [21] detected very low levels of corticosterone in serum during stages V to X, and then serum corticosterone concentration increased steadily until stage XVII. In the present study, which examined the effects of corticosterone and cortisol in bullfrog tadpoles (stages VII-XII) kept in low-sodium water, the hypernatremic effect of corticosterone did not exceed that of cortisol at the same dose. It is not yet known whether cortisol is produced in larval amphibians. However, according to Krug *et al.* [21] a cortisol-like substance is present in the serum of young tadpoles (stages V-XXV). In the present study, cortisol was the most potent hypernatremic agent in young tadpoles. In pre-metamorphic bullfrog tadpoles, a cortisol-like substance was detectable [21] and their gills were important site for sodium uptake [4, 10]. Therefore, a cortisol-like substance might be involved in the uptake of sodium by the gills of bullfrog tadpoles. This may also influence the metabolic processes in the body, thus indirectly affecting sodium homeostasis and osmoregulation. In conclusion, it seems that the pituitary-adrenal axis is important for plasma sodium regulation in pre-metamorphic tadpoles.

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